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EXAMINER	
MUMMERT, STEPHANIE KANE	
ART UNIT	**PAPER NUMBER**
1637	

DATE MAILED: 01/25/2006

Please find below and/or attached an Office communication concerning this application or proceeding.

Office Action Summary	Application No.	Applicant(s)	
	10/684,205	CHRISTIANS ET AL.	
	Examiner	Art Unit	
	Stephanie K. Mummert	1637	

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE 3 MONTH(S) OR THIRTY (30) DAYS, WHICHEVER IS LONGER, FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☐ Responsive to communication(s) filed on _____.

2a)☐ This action is **FINAL**. 2b)☒ This action is non-final.

3)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

4)☒ Claim(s) *1-43* is/are pending in the application.

4a) Of the above claim(s) *27 and 29* is/are withdrawn from consideration.

5)☐ Claim(s) _____ is/are allowed.

6)☒ Claim(s) *1-26,28 and 30-43* is/are rejected.

7)☐ Claim(s) _____ is/are objected to.

8)☐ Claim(s) _____ are subject to restriction and/or election requirement.

Application Papers

9)☐ The specification is objected to by the Examiner.

10)☐ The drawing(s) filed on _____ is/are: a)☐ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).

11)☐ The oath or declaration is objected to by the Examiner. Note the attached Office Action or form PTO-152.

Priority under 35 U.S.C. § 119

12)☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

a)☐ All b)☐ Some * c)☐ None of:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____.

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

Attachment(s)

1) ☒ Notice of References Cited (PTO-892)
2) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)
3) ☒ Information Disclosure Statement(s) (PTO-1449 or PTO/SB/08) Paper No(s)/Mail Date 7/12/04
4) ☐ Interview Summary (PTO-413) Paper No(s)/Mail Date. _____.
5) ☐ Notice of Informal Patent Application (PTO-152)
6) ☐ Other: _____.

DETAILED ACTION

The preliminary amendment filed May 17, 2004 amending claims 24, 30, 31-33, canceling claim 27 and 29, and adding claims 37-43 is acknowledged and has been entered. Claims 1-26, 28 and 30-43 are pending and will be examined.

Information Disclosure Statement

1. The information disclosure statement (IDS) submitted on July 12, 2004 is in compliance with the provisions of 37 CFR 1.97. Accordingly, the information disclosure statement is being considered by the examiner.

Claim Rejections - 35 USC § 112

2. The following is a quotation of the second paragraph of 35 U.S.C. 112:

> The specification shall conclude with one or more claims particularly pointing out and distinctly claiming the subject matter which the applicant regards as his invention.

3. Claim 40 recites the limitation "said one or more blocking molecules are peptide nucleic acids" in the body of the claim. As currently written, the claim depends from claim 38, where there is no mention of "blocking molecules". There is insufficient antecedent basis for this limitation in the claim.

Claim Interpretation

The terms "reduction oligonucleotide" and "blocking oligonucleotide" are being given the broadest reasonable interepretation in light of the specification. The terms are used interchangeably within the claims and the term "reduction oligonucleotide" is defined within the

specification as "an oligonucleotide that is complementary to an unwanted nucleic acid. For example, SEQ ID NOs 1, 2 and 3 may be used as reduction oligos targeting unwanted globin mRNAs" (p. 10, lines 1-3). The term is being interpreted to broadly include any oligonucleotide complementary to an unwanted nucleic acid.

Claim Rejections - 35 USC § 102

4. The following is a quotation of the appropriate paragraphs of 35 U.S.C. 102 that form the basis for the rejections under this section made in this Office action:

> A person shall be entitled to a patent unless –
>
> (b) the invention was patented or described in a printed publication in this or a foreign country or in public use or on sale in this country, more than one year prior to the date of application for patent in the United States.
>
> (e) the invention was described in (1) an application for patent, published under section 122(b), by another filed in the United States before the invention by the applicant for patent or (2) a patent granted on an application for patent by another filed in the United States before the invention by the applicant for patent, except that an international application filed under the treaty defined in section 351(a) shall have the effects for purposes of this subsection of an application filed in the United States only if the international application designated the United States and was published under Article 21(2) of such treaty in the English language.

5. Claims 37-38 and 42 are rejected under 35 U.S.C. 102(b) as being anticipated by Lockhart et al. (Nat Biotech, 1996, vol. 14, p. 1675-1680). Lockhart teaches hybridization to small, high-density oligonucleotide arrays, designed using a combination of photolithography and oligonucleotide synthesis (Abstract).

With regard to claim 37, Lockhart discloses a method of analyzing a nucleic acid sample from a blood sample comprising:

a) amplifying mRNA from the nucleic acid sample to generate an amplified sample (Figure 1; p. 1679, col. 1 'RNA preparation for hybridization' heading);

b) labeling said amplified sample (Figure 1; p. 1679, col. 1 'RNA preparation for hybridization' heading);

c) hybridizing the amplified sample to an array of nucleic acid probes to generate a hybridization pattern (Figure 1; p. 1679, col. 2, 'array hybridization and scanning'); and

d) analyzing the hybridization pattern (Figure 1; p. 1679, col. 2 'quantitative analysis of hybridization patterns and intensities' heading).

With regard to claim 38, Lockhart discloses an embodiment of claim 37, wherein said amplifying step comprises hybridizing an extendable primer comprising oligo dT to said nucleic acid sample, extending said primer with a reverse transcriptase to make cDNA and amplifying said cDNA (p. 1679, col. 1, 'RNA preparation for hybridization' heading, where 1 microgram of poly (A)+ RNA was converted into double stranded cDNA using a cDNA synthesis kit with an oligo dT primer incorporating a T7 RNA polymerase promoter site).

With regard to claim 42, Lockhart discloses an embodiment of claim 37, wherein the hybridization pattern is analyzed to determine an expression profile for said nucleic acid sample (Figure 1 and 2, p. 1679, col. 2 'Higher density arrays containing 65,000 probes for over 100 murine genes' heading, paragraph 2, where experiments were conducted to determine the proper number of redundantly spotted oligonucleotides necessary for efficient measurement of expression changes at low levels).

Claim Rejections - 35 USC § 103

6. The following is a quotation of 35 U.S.C. 103(a) which forms the basis for all obviousness rejections set forth in this Office action:

> (a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negatived by the manner in which the invention was made.

7. Claims 1-2, 4 are rejected under 35 U.S.C. 103(a) as being anticipated by Mugasimangalam et al. (US Patent 6,544,741; September 2003) in view of Kempe et al. (US Patent 4,661,450; April 1987). Mugasimangalam discloses methods and materials for normalizing nucleic acids, using a technique called 'prime and kill' (col. 3, lines 44-51).

With regard to claim 1, Mugasimangalam discloses a method for amplifying a nucleic acid sample from blood comprising the steps:

a) hybridizing at least one reduction oligonucleotide to at least one unwanted RNA in the sample (col. 3, lines 52-57, where a "killer primer" is designed complementary to the 3' end of highly and/or moderately expressed genes; example 1, col. 13, lines 17-22);

b) incubating the mixture with an RNAse H and subsequently inactivating the RNAse H (col. 3, lines 57-58, where RNAse H is used to digest the RNA in the DNA:RNA duplex; col. 5, lines 20-25; example 1, col. 13, lines 17-22, where the RNAse H is inactivated by heating);

d) extending a primer to make cDNA (col. 3, lines 61-62, where cDNA is synthesized from oligo dT primer; example 1, col. 13, lines 25-35); and

e) amplifying the cDNA (col. 4, lines 21-24, where an embodiment includes testing the efficiency of killing by PCR amplification; col. 5, lines 20-25).

With regard to claim 2, Mugasimangalam discloses an embodiment of claim 1, wherein the unwanted RNA comprises a poly(A) tail and wherein the reduction oligonucleotide hybridizes to the unwanted RNA in the region of the unwanted RNA that is near the 5' end of the

poly(A) tail of the unwanted RNA (Figure 1A, where the reduction oligo hybridizes near the poly(A) tail; col. 3, lines 52-57, where a "killer primer" is designed complementary to the 3' end of highly and/or moderately expressed genes).

With regard to claim 4, Mugasimangalam discloses an embodiment of claim 1, wherein the RNAse H is thermolabile and inactivation is by heating (col. 5, lines 20-25).

Regarding claim 1, Mugasimangalam does not specifically teach that the nucleic acid sample is obtained from a blood sample. Kempe teaches the extraction of RNA from blood obtained from rabbit reticulocytes, establishing that blood can be a good source of RNA, and particularly of the globin sequence (col. 27, lines 39-60).

It would have been prima facie obvious to one of ordinary skill in the art at the time the invention was made to extend the technique taught by Mugasimangalam, disclosed as capable of using virtually any prokaryotic or eukaryotic cell, to include extraction of nucleic acids from blood samples. As taught by Mugasimangalam, the technique "allows preferential elimination of highly expressed genes through cycling of the killing reaction" and "when used for subtraction, this embodiment allows enrichment of differentially expressed genes by degrading other mRNAs through killing reactions" (col. 4, lines 57-63). Mugasimangalam also teaches that "nucleic acid molecule comprises also any feasible derivative of a nucleic acid to which a nucleic acid probe can hybridize" (col. 7, lines 41-51). One of ordinary skill in the art would have recognized the universal nature of the technique taught by Mugasimangalam and also would have recognized the teaching that the method could be applicable to any cell type, and would therefore have been motivated to apply the technique to any tissue type of interest, including blood samples and tissues of interest, with a reasonable expectation for success.

8. Claims 3 and 6 are rejected under 35 U.S.C. 103(a) as being unpatentable over Mugasimangalam et al. (US Patent 6,544,741; September 2003) in view of Kempe et al. (US Patent 4,661,450; April 1987) as applies to claims 1-2 and 4 above and further in view of Rabin et al. (US Patent 4,745,054; May 1988). Mugasimangalam discloses methods and materials for normalizing nucleic acids, using a technique called 'prime and kill' (col. 3, lines 44-51).

Mugasimangalam in view of Kempe discloses the limitations of claims 1-2 and 4 as recited in the 103 rejection stated above. Mugasimangalam teaches the inactivation of RNAse H with heat, after the digestion of the RNA portion of the RNA:DNA hybrid; however, Mugasimangalam does not disclose the limitations of claims 3 or 6, where the ribonuclease is inactivated through organic extraction.

Rabin discloses a method of detecting an analyte in a sample after an enzymatic reaction (Abstract).

With regard to claim 3, Rabin discloses an embodiment of claim 1, wherein the RNAse H is inactivated by depleting RNAse H from the mixture (experimental section, II. 'stopping the reaction' heading, col. 11, lines 31-46).

With regard to claim 6, Rabin discloses an embodiment of claim 1, wherein the RNAse H is inactivated by separating the RNAse H from the nucleic acid by organic extraction (experimental section, II. 'stopping the reaction' heading, col. 11, lines 31-46).

It would have been prima facie obvious to one of ordinary skill in the art at the time the invention was made to extend the inactivation of RNAse H taught by Mugasimangalam by using alternate techniques known in the art at the time the invention was made. These techniques are

varied and are designed to remove or inactivate an enzyme that may interfere with downstream steps after reaction using the enzyme (e.g., ribonuclease). Rabin teaches of depletion of a ribonuclease after conducting a step directed to Enzyme catalysed production of Cp Riboflavin using phenol extraction. While the method taught by Rabin uses a ribonuclease as part of a large enzymatic reaction, the concept taught by Rabin of the necessity of stopping the reaction is shared between Rabin and Mugasimangalam. As taught by Rabin, "It was essential at this point to inactivate the ribonuclease in the incubation mixture as exposure of the product to the enzyme in an aqueous environment would result in its breakdown to cytidine 3'-phosphate and riboflavin" (col. 11, lines 31-46). While the ribonuclease taught by Mugasimangalam is used to digest RNA, it is important to inactivate the enzyme before further steps are conducted to avoid digestion of non-targeted sequences that remain in the sample following reaction with the blocking oligonculeotide. One of ordinary skill in the art at the time the invention was made would have been motivated to consider alternate techniques to deplete, remove or inactivate the RNAse H enzyme prior to further processing of the mRNA sample with a reasonable expectation of success.

9. Claims 3, 5 and 7 are rejected under 35 U.S.C. 103(a) as being unpatentable over Mugasimangalam et al. (US Patent 6,544,741; September 2003) in view of Kempe et al. (US Patent 4,661,450; April 1987) as applies to claims 1-2 and 4 above and further in view of Stamatoyannapoulos et al. (2003/0170689; September 2003). Mugasimangalam discloses methods and materials for normalizing nucleic acids, using a technique called 'prime and kill' (col. 3, lines 44-51).

With regard to claim 3, Stamatoyannapoulos discloses an embodiment of claim 1, wherein the RNAse H is inactivated by depleting RNAse H from the mixture (p. 18, paragraph 0188, where reaction was 'cleaned up' using a column to remove enzymes, etc. from a reaction used to blunt a DNA molecule).

With regard to claim 5, Stamatoyannapoulos discloses an embodiment of claim 1, wherein the RNAse H is inactivated by addition of EDTA to the mixture (p. 17, paragraph 0155, example 7, where EDTA is added to quench a reaction).

With regard to claim 7, Stamatoyannapoulos discloses an embodiment of claim 1, wherein the RNAse H is removed by separating the RNA from the RNAse H by column purification (p. 18, paragraph 0188, where reaction was 'cleaned up' using a column to remove enzymes, etc. from a reaction used to blunt a DNA molecule).

It would have been prima facie obvious to one of ordinary skill in the art at the time the invention was made to extend the inactivation of RNAse H taught by Mugasimangalam by using alternate techniques known in the art at the time the invention was made. These techniques are varied and are designed to remove or inactivate an enzyme that may interfere with downstream steps after reaction using the enzyme (e.g., Klenow or polymerase). Stamatoyannapoulos teaches multiple instances of inactivation or depletion of enzyme(s) following particular steps in a multi-step protocol. The method taught by Stamatoyannapoulos, includes multiple steps, including labeling of DNA fragments using a Klenow fragment, followed by stopping the reaction by the addition of EDTA (p. 17, paragraph 0155) and a 'clean up' step after blunting and tailing probes for hybridization to a microarray by processing on a column (p. 18, paragraph 0188). While neither of these steps directly involves the inactivation or depletion of a

ribonuclease, such as RNAse H, the steps demonstrate the state of the art with regards to different techniques directed to inactivation or depletion of enzymes following an enzymatic reaction. The ribonuclease of Mugasimangalam is used to digest RNA, it is important to inactivate the enzyme before further steps are conducted to avoid digestion of non-targeted sequences that remain in the sample following reaction with the blocking oligonculeotide. One of ordinary skill in the art at the time the invention was made would have been motivated to consider alternate techniques, such as those taught by Stamatoyannapoulos to deplete, remove or inactivate the an enzyme such as RNAse H prior to further processing of the mRNA sample with a reasonable expectation of success.

10. Claims 8 and 9 are rejected under 35 U.S.C. 103(a) as being unpatentable over Mugasimangalam et al. (US Patent 6,544,741; September 2003) in view of Kempe et al. (US Patent 4,661,450; April 1987) as applies to claims 1-2 and 4 above and further in view of Kwoh et al. (US Patent 5,055,393; October 1991). Mugasimangalam discloses methods and materials for normalizing nucleic acids, using a technique called 'prime and kill' (col. 3, lines 44-51).

Mugasimangalam in view of Kempe teaches the limitations of claims 1-2 and 4 as recited in the 103 rejection stated above. However, neither Mugasimangalam nor Kempe disclose the inclusion of an RNA promoter sequence in the primer used to prime cDNA synthesis.

Kwoh teaches the inclusion of an RNA promoter sequence in the primer used to prime cDNA synthesis. Kwoh discloses methods for determining the sex of bovine embryos (Abstract).

With regard to claim 8, Kwoh discloses an embodiment of claim 1, wherein the primer further comprises an RNA polymerase promoter sequence (col. 9, lines 34-51, where a primer A including a T7 promoter is included).

With regard to claim 9, Kwoh discloses an embodiment of claim 8, wherein the step of amplifying the cDNA comprises making double stranded cDNA comprising a functional RNA polymerase promoter region and synthesizing multiple copies of RNA from the double stranded cDNA using an RNA polymerase (col. 9, lines 34-51, where a primer A including a T7 promoter is included and the primer B is used to initiate synthesis of the second strand of cDNA; incubation with T7 polymerase results in the synthesis of RNA transcripts from the cDNA).

It would have been prima facie obvious to one of ordinary skill in the art at the time the invention was made to include the method of transcription amplification system (TAS) taught by Kwoh into the method of sequence analysis taught by Mugasimangalam with a reasonable expectation for success. As taught by Kwoh, "Although target amplification is illustrated by the PCR amplification procedure, other target amplification techniques, for example the so called transcription amplification system (TAS), may equally be employed." As further noted by Kwoh, "incubation of the double stranded cDNA with T7 RNA polymerase and ribonucleotide triphosphates will result in the synthesis of RNA transcripts from the cDNA. Additional amplification can be achieved by repeating TAS on the newly synthesized RNA" (col. 10, lines 24-27 and lines 47-51). The method taught by Mugasimangalam incorporates a step of PCR amplification to determine the level or efficiency of "killing" by the stated method and by the same reasoning stated by Kwoh, it would have been obvious to include alternate amplification techniques to amplify the non-targeted sequences that remain in the sample following the

"killing" or reduction step. One of ordinary skill in the art would have recognized the multiple types of amplification techniques available and would have been motivated to incorporate additional amplification techniques, in addition to the PCR amplification taught by Mugasimangalam, including the technique disclosed by Kwoh, with a reasonable expectation for success.

11. Claims 10-12 are rejected under 35 U.S.C. 103(a) as being unpatentable over Mugasimangalam et al. (US Patent 6,544,741; September 2003) in view of Kempe et al. (US Patent 4,661,450; April 1987) as applies to claims 1-2 and 4 above further in view of Baker et al. (US Patent 5,643,780; July 1997). Mugasimangalam discloses methods and materials for normalizing nucleic acids, using a technique called 'prime and kill' (col. 3, lines 44-51).

Mugasimingalam in view of Kempe discloses the details of claims 1-2 and 4 as recited in the 103 rejection stated above. However, Mugasimingalam does not teach the targeting of globin sequences specifically. Baker teaches that overabundance of specific highly expressed sequences in the construction of libraries can be addressed through the inactivation and degradation of overabundant mRNA species (col. 5, lines 5-24).

With regard to claim 10, Baker discloses an embodiment of claim 1, wherein the unwanted nucleic acid is a globin mRNA (col. 5, lines 5-24).

With regard to claim 12, Baker discloses an embodiment of claim 10, wherein a plurality of different species of reduction oligonucleotides are used and each species is complementary to a globin mRNA (col. 5, lines 5-24).

Baker does not teach specific subtypes of globin sequences, however Kempe discloses the isolation of globin from blood and teaches the isolation of a specific subtype of globin.

With regard to claim 11, Kempe discloses an embodiment of claim 1, wherein the unwanted nucleic acid is selected from the group consisting of alpha-1 globin, alpha-2-globin and beta globin (col. 27, lines 39-60, where beta globin is extracted from blood).

It would have been prima facie obvious to one of ordinary skill in the art at the time the invention was made to have included the abundantly expressed globin gene taught by Baker in addition to the sequences that were targeted by Mugasimangalam with a reasonable expectation for success. As taught by Mugasimangalam, "the methods and materials provided herein efficiently reduce the redundancy of highly expressed genes, and increase the relative amount of transcripts of rarely expressed genes" (col. 7, lines 5-13). As noted by Baker, "A common problem encountered is overabundance of a particular undesired message in the library. In many cells or tissues, a particular mRNA species represents the vast majority of the total mRNA. For example, abundant mRNAs, such as those encoding globin, immunoglobulins and ovalbumin may constitute as much as 50-90% of the total poly(A)+ cytoplasmic RNA isolated from certain cell types (col. 5, lines 5-12)." Considering the abundance of expression of globin in certain cell types, it would have been prima facie obvious to include this target to decrease the abundance of this target gene in the method of enrichment for less highly expressed sequences in a complex mixture of nucleic acids. Given the teachings of both Baker and Mugasimangalam, one of ordinary skill would have recognized the benefit of greater representation of less highly expressed genes in a complex mixture of nucleic acids and would therefore have been motivated to incorporate the globin sequence taught by Baker into the preparation of the nucleic acid

molecules applied to the hybridization assay taught by Lockhart with a reasonable expectation for success.

12. Claims 13 and 31-34 are rejected under 35 U.S.C. 103(a) as being unpatentable over Mugasimangalam et al. (US Patent 6,544,741; September 2003) in view of Kempe et al. (US Patent 4,661,450; April 1987) as applies to claims 1-2 and 4 above and further in view of Baker et al. (US Patent 5,643,780; July 1997) and Rampersad et al. (US Patent 5,830,712; November 1998). Mugasimangalam discloses methods and materials for normalizing nucleic acids, using a technique called 'prime and kill' (col. 3, lines 44-51).

Mugasimingalam in view of Kempe discloses the details of claims 1-2 and 4 as recited in the 103 rejection stated above. However, Mugasimingalam does not teach the targeting of globin sequences specifically. Baker teaches that overabundance of specific highly expressed sequences in the construction of libraries can be addressed through the inactivation and degradation of overabundant mRNA species (col. 5, lines 5-24).

With regard to claim 11, Kempe discloses an embodiment of claim 1, wherein the unwanted nucleic acid is selected from the group consisting of alpha-1 globin, alpha-2-globin and beta globin (col. 27, lines 39-60, where beta globin is extracted from blood).

Mugasimingalam does not teach the extension of the reduction oligonucleotide by a polymerase, or the removal of the reduction oligonucleotide after reaction with the target nucleic acid as recited in claims 13-14 and 31-34.

Rampersad teaches a method for inactivating undesirable members in a sample which includes desirable and undesirable sequences in a mixture (Abstract), including these steps, as follows.

With regard to claim 13, Rampersad discloses an embodiment of claim 11, wherein after hybridizing the reduction oligonucleotide to the unwanted mRNA, the reduction oligonucleotide is extended by a polymerase (example 1, col. 5, lines 9-61, where RNA treated with the oligonucleotide blocker plus or minus oligonucleotide blocker and RNAse H were used as templates in reverse transcription reactions to generate cDNA, with the oligonucleotide blocker priming cDNA synthesis).

With regard to claim 14, Rampersad discloses an embodiment of claim 1, wherein after incubating the mixture with RNAse H, the reduction oligonucleotide is removed (col. 6, lines 10-23).

With regard to claim 31, Rampersad discloses a method for amplifying a nucleic acid sample comprising:

a) providing a nucleic acid sample (col. 4, lines 1-17; col. 5, lines 9-33);

b) hybridizing at least one reduction oligonucleotide to at least one mRNA in the sample generating reduction oligonucleotide:mRNA complexes (col. 4, lines 1-17, where the DNA:RNA hybrids form between the mRNA and the oligo blocker; example 1, col. 5, lines 9-33, where an oligonucleotide blocker specific to rat NPY Y1 sequence was incubated with total rat hypothalamus RNA);

c) removing said complexes from the sample (col. 6, lines 10-23); and

d) amplifying at least one target RNA remaining in the sample (example 1, col. 5, lines 48-61, where cDNA was used in subsequent PCR reactions).

With regard to claim 32, Rampersad discloses an embodiment of claim 31, wherein said complexes are removed from the sample by affinity purification (col. 6, lines 10-23).

With regard to claim 33, Rampersad discloses an embodiment of claim 31, wherein said reduction oligonucleotide comprises biotin and said complexes are removed from the sample by hybridization to a solid support (col. 6, lines 10-23, where the oligonucleotide blocker is removed using biotin/streptavidin bead technology).

With regard to claim 34, Rampersad discloses an embodiment of claim 33, wherein said solid support comprises streptavidin (col. 6, lines 10-23, where the oligonucleotide blocker is removed using biotin/streptavidin bead technology).

It would have been prima facie obvious to one of ordinary skill in the art at the time the invention was made to incorporate the method of inactivation and blocking taught by Rampersad to complement the "killer primer" method taught by Mugasimangalam with a reasonable expectation for success. As taught by Rampersad, the term "undesired nucleic acids is used herein to denote nucleic acids in a sample that interfere with the use of the sample. Undesirable nucleic acids may include known or abundant members of a nucleic acid family that block the study, isolation or processing of desired novel or less abundant members of the same family" (col. 2, lines 30-34). While, Mugasimangalam teaches that "the methods and materials provided herein efficiently reduce the redundancy of highly expressed genes, and increase the relative amount of transcripts of rarely expressed genes" (col. 7, lines 5-13). As both Rampersad and Mugasimangalam teach related methods for the reduction of abundant target sequences in

complex mixtures of oligonucleotides, it would have been obvious to one of ordinary skill in the art at the time the invention was made to incorporate the teachings of Rampersad, including the removal of the blocking oligonucleotide following reaction with the nucleic acid sample and potentially designing the killer primer to be extendable by a polymerase, instead of priming synthesis using oligo d(T), as taught by Mugasimangalam. One of ordinary skill would have recognized the benefit of modifying a technique to fit a given set of experimental conditions and would have therefore been motivated to consult similar methods in the art in order to arrive at the most optimal experimental conditions to achieve the stated goal.

13. Claims 15-19 are rejected under 35 U.S.C. 103(a) as being unpatentable over Mugasimangalam et al. (US Patent 6,544,741; September 2003) in view of Kempe et al. (US Patent 4,661,450; April 1987) as applies to claims 1-2, 4 above and further in view of Baker (US Patent 5,643,780; July 1997) and Adams et al. (Nature, 1995, supplement, vol. 377, p. 3-17). Mugasimangalam discloses methods and materials for normalizing nucleic acids, using a technique called 'prime and kill' (col. 3, lines 44-51).

The "consisting essentially of" language used as a transitional phrase in these claims is being interpreted as "comprising" language. As noted in the MPEP, "For the purposes of searching for and applying prior art under 35 U.S.C. 102 and 103, absent a clear indication in the specification or claims of what the basic and novel characteristics actually are, "consisting essentially of" will be construed as equivalent to "comprising." See, e.g., PPG, 156 F.3d at 1355, 48 USPQ2d at 1355.

Mugasimangalam in view of Kempe discloses the limitations of claims 1-2 and 4 as recited above. With regard to claim 18, Mugasimangalam discloses an embodiment of claim 1, wherein a mixture of different sequence reduction oligonucleotides are added to the mixture (col. 15, lines 1-49, where mixtures of killer primers targeting different genes, or families of genes are disclosed, acting specifically in rat liver). However, Mugasimangalam does not teach specific sequences directed to globin sequences. Baker teaches that overabundance of specific highly expressed sequences in the construction of libraries can be addressed through the inactivation and degradation of overabundant mRNA species (col. 5, lines 5-24). Baker also does not teach specific sequences targeting globin molecules.

With regard to claim 15, Adams discloses an embodiment of claim 1, wherein the at least one reduction oligonucleotide consists essentially of SEQ ID NO:1 (attached sequence results, entry 4, where AA342142 discloses the 3' end of a cDNA sequence obtained from spleen which is similar to alpha 1 globin, with sequence identity of 100% across the 23 nucleotides comprising SEQ ID NO:1; see also p. 12, Table 11, of widely expressed genes, including globin).

With regard to claim 16, Adams discloses an embodiment of claim 1, wherein the at least one reduction oligonucleotide consists essentially of SEQ ID NO:2 (attached sequence results, entry 4, where AA342142 discloses the 3' end of a cDNA sequence obtained from liver which is similar to alpha 2 globin, with sequence identity of 100% across the 23 nucleotides comprising SEQ ID NO:2; see also p. 12, Table 11, of widely expressed genes, including globin).

With regard to claim 17, Adams discloses an embodiment of claim 1, wherein the at least one reduction oligonucleotide consists essentially of SEQ ID NO:3 (attached sequence results, entry 4, where AA342142 discloses the 3' end of a cDNA sequence obtained from spleen which

is similar to beta globin, with sequence identity of 100% across the 26 nucleotides comprising SEQ ID NO:3; see also p. 12, Table 11, of widely expressed genes, including globin).

With regard to claim 19, Adams discloses an embodiment of claim 18, wherein the mixture comprises SEQ ID NO: 1, 2 and 3 (see attached results, where sequences comprising alpha 1 globin (SEQ ID 1), alpha 2 globin (SEQ ID 2) and beta globin (SEQ ID 3) are disclosed by Adams).

It would have been prima facie obvious to one of ordinary skill in the art at the time the invention was made to substitute the oligonucleotides designed to target a highly expressed gene such as globin, comprising SEQ ID NO: 1, 2 and 3 as taught by Adams for the oligonucleotide "killer primers" taught by Mugasimangalam, with a reasonable expectation for success. As taught by Baker, "A common problem encountered is overabundance of a particular undesired message in the library. In many cells or tissues, a particular mRNA species represents the vast majority of the total mRNA. For example, abundant mRNAs, such as those encoding globin, immunoglobulins and ovalbumin may constitute as much as 50-90% of the total poly(A)+ cytoplasmic RNA isolated from certain cell types (col. 5, lines 5-12)." Considering the abundance of expression of globin in certain cell types, it would have been prima facie obvious to include this target to decrease the abundance of this target gene in the method of "killer primer" enrichment for less highly expressed sequences in the construction of a cDNA library. Given the teachings of both Baker and Mugasimangalam, one of ordinary skill would have recognized the benefit of greater representation of less highly expressed genes in the formation of cDNA libraries and would therefore have been motivated to incorporate the globin sequence

taught by Adams into the reduction oligonucleotide or "killer primer" technique taught by Mugasimangalam with a reasonable expectation for success.

14. Claims 20-23 are rejected under 35 U.S.C. 103(a) as being obvious over Mugasimangalam et al. (US Patent 6,544,741; September 2003) in view of Kempe et al. (US Patent 4,661,450; April 1987) as applies to claims 1-2 and 4 above and further in view of Augello et al. (US Patent 6,602,718; August 2003). Mugasimangalam discloses methods and materials for normalizing nucleic acids, using a technique called 'prime and kill' (col. 3, lines 44-51).

Mugasimangalam teaches the limitations of claims 1-2 and 4 as recited in the 103 rejection stated above. However, Mugasimangalam does not specifically teach that the nucleic acid sample is isolated from blood or that the blood was collected in a container containing an RNA stabilizing agent. Augello teaches a container specifically designed for the collection of blood and includes a variety of RNA stabilizing agents (Abstract; col. 3, lines 44-58).

With regard to claim 20, Augello discloses an embodiment of claim 1, wherein said nucleic acid sample from blood is obtained from blood that was collected in a container containing an RNA stabilizing agent (Abstract; col. 3, lines 44-58).

With regard to claim 21, Augello discloses an embodiment of claim 20, wherein said RNA stabilizing agent is selected from the group consisting of cationic compounds, detergents, chaotropic salts, ribonuclease inhibitors, chelating agents, and mixtures thereof (col. 3, lines 44-

58, where the nucleic acid stabilizing agent can be a detergent, a chaotropic salt, RNAse inhibitors, chelating agents, or mixtures; see also col. 6, line 43 to col. 8, line 59).

With regard to claim 22, Augello discloses an embodiment of claim 20, wherein said RNA stabilizing agent is selected from the group consisting of phenol, chloroform, acetone, alcohols and mixtures thereof (col. 6, line 51 to col. 7 line 18).

With regard to claim 23, Augello discloses an embodiment of claim 20, wherein said nucleic acid sample from blood is obtained from blood that was collected in a container containing an RNA stabilizing agent and wherein said RNA stabilizing agent is selected from the group consisting of mercapto-alcohols, dithiothreitol (DTT) and mixtures thereof (col. 6, line 51 to col. 7 line 18).

It would have been prima facie obvious to one of ordinary skill in the art at the time the invention was made to incorporate the teachings of Augello into the method taught by Mugasimangalam with a reasonable expectation for success. As taught by Augello, upon evaluating the stabilization of nucleic acids obtained using the collection container with RNA stabilizers, "The real-time PCR results show that in the unpreserved EDTA blood, the transcript level decreases over time (indicated by the increasing Ct value in the TaqMan analysis) up to a degree of degradation after 7 to 10 days at which point the mRNA is no longer detectable. On the other hand the GAPDH mRNA in the preserved samples does not show any decrease in copy number" (col. 11, lines 26-33). Therefore, the inclusion of the RNA stabilizer and collection container taught by Augello provides the benefit of increased stability over time, allowing for analysis of RNA samples over a longer time frame. One of ordinary skill in the art would have recognized the benefit of blood collection in the presence of RNA stabilizers, who would have

therefore been motivated to incorporate the teachings of Augello to the method taught by Mugasimangalam with a reasonable expectation for success.

15. Claims 24-26, 28, 30-31 and 35-36 are rejected under 35 U.S.C. 103(a) as being unpatentable over Mugasimangalam et al. (US Patent 6,544,741; September 2003) in view of Kempe et al. (US Patent 4,661,450; April 1987) as applied to claims 1-2 and 4 above, and further in view of Lockhart et al. (1996, Nature Biotech, vol. 14, p. 1675-1680). Mugasimangalam discloses methods and materials for normalizing nucleic acids, using a technique called 'prime and kill' (col. 3, lines 44-51).

With regard to claim 24, Mugasimangalam discloses a method for analyzing a nucleic acid sample comprising:

a) providing a first nucleic acid sample (col. 3, lines 52-57; col. 11, lines 5-10, where the biological sample is from an animal, plant, bacterium or virus or can be from any eukaryotic or prokaryotic cell);

b) blocking amplification of sequences in the nucleic acid sample by hybridizing a reduction oligonucleotide to said mRNA sequences to form an RNA:DNA hybrid and digesting the RNA:DNA hybrids (col. 3, lines 52-57, where a "killer primer" is designed complementary to the 3' end of highly and/or moderately expressed genes; example 1, col. 13, lines 17-22; col. 3, lines 57-58, where RNAse H is used to digest the RNA in the DNA:RNA duplex; col. 5, lines 20-25; example 1, col. 13, lines 17-22);

c) amplifying unblocked nucleic acid sequences to produce an amplified nucleic acid sample (col. 4, lines 21-24, where an embodiment includes testing the efficiency of killing by PCR amplification; col. 5, lines 20-25);

With regard to claim 30, Mugasimangalam discloses an embodiment of claim 24, wherein said target mRNAs are greater than 20% of the first nucleic acid sample and wherein said target mRNAs are less than 20% of the amplified nucleic acid sample (col. 6, lines 5-13, where the target genes comprise 0.5 to 90% pf all of the genes in the sample; col. 14, lines 5-7, where targeted high copy number genes were not detected after treatment with the "killer primer" method).

With regard to claim 31, Mugasimangalam discloses a method for amplifying a nucleic acid sample comprising:

a) providing a nucleic acid sample (col. 3, lines 52-57; col. 11, lines 5-10, where the biological sample is from an animal, plant, bacterium or virus or can be from any eukaryotic or prokaryotic cell);

b) hybridizing at least one reduction oligonucleotide to at least one mRNA in the sample generating reduction oligonucleotide:mRNA complexes (col. 3, lines 52-57, where a "killer primer" is designed complementary to the 3' end of highly and/or moderately expressed genes; example 1, col. 13, lines 17-22);

c) removing said complexes from the sample (col. 3, lines 57-58, where RNAse H is used to digest the RNA in the DNA:RNA duplex; col. 5, lines 20-25; example 1, col. 13, lines 17-22); and

d) amplifying at least one target RNA remaining in the sample (col. 4, lines 21-24, where an embodiment includes testing the efficiency of killing by PCR amplification; col. 5, lines 20-25).

With regard to claim 35, Mugasimangalam discloses an embodiment of claim 31, wherein the RNA is amplified by mixing with random primers, extending the random primers to make cDNA (col. 4, lines 39-42, where the cDNA is primed using random primers or oligo d(T) for first strand synthesis).

Regarding claim 24 and 31, Mugasimangalam does not specifically teach obtaining the nucleic acid sample from blood, targeting of globin mRNA specifically in the complex mixture and also does not teach contacting the amplified nucleic acid sample with a solid support to generate a hybridization pattern. Mugasimangalam also does not teach the labeling of the cDNA.

Baker teaches that overabundance of specific highly expressed sequences in the construction of libraries can be addressed through the inactivation and degradation of overabundant mRNA species (col. 5, lines 5-24). Baker also teaches that a specific example of an overexpressed gene includes globin (col. 5, lines 5-24).

Neither Mugasimangalam or Baker teach the hybridization of nucleic acids to a solid support. Lockhart teaches the hybridization of nucleic acids to a solid support to generate a hybridization pattern to determine expression levels (Abstract).

With regard to claim 25, Lockhart discloses an embodiment of claim 24, further comprising detecting the presence or absence of hybridization of said amplified nucleic acid sample to said nucleic acid probes on said solid support (Figure 1, where labeled transcripts are hybridized to array; p 1679, col. 2 'array hybridization and scanning' heading).

With regard to claim 26, Lockhart discloses an embodiment of claim 24, further comprising labeling said amplified nucleic acid sample (Figure 1, where the transcripts are labeled during in vitro transcription; p. 1679, col. 1 'RNA preparation for hybridization', where labeled cRNA was made directly from the cDNA pool with an in vitro transcription step).

With regard to claim 28, Lockhart discloses an embodiment of claim 24, wherein said unblocked nucleic acid sequences are non-specifically amplified by in vitro transcription (Figure 1, where the transcripts are labeled during in vitro transcription; p. 1679, col. 1 'RNA preparation for hybridization', where labeled cRNA was made directly from the cDNA pool with an in vitro transcription step).

With regard to claim 35, Lockhart discloses that the cDNA is labeled (Figure 1, where the transcripts are labeled during in vitro transcription; p. 1679, col. 1 'RNA preparation for hybridization', where labeled cRNA was made directly from the cDNA pool with an in vitro transcription step).

With regard to claim 36, Lockhart discloses an embodiment of claim 35, wherein the labeled cDNA is hybridized to a solid support and the hybridization pattern is analyzed (Figure 1, where labeled transcripts are hybridized to array; p 1679, col. 2 'array hybridization and scanning' heading).

It would have been prima facie obvious to one of ordinary skill in the art at the time the invention was made to analyze the amplified sample, enriched for less abundant transcripts, through hybridization to an oligonucleotide array. According to Lockhart, the technique of array hybridization provides the benefit that "oligonucleotide probes are specifically chosen and synthesized in known locations on the arrays" and "hybridization patterns and intensities can be

interpreted in terms of gene identity and relative amount with no additional sequencing or characterization" (p. 1676, col. 2, top paragraph). Furthermore, the technique of array hybridization has the added quality that "in addition to being sensitive, specific and quantitative, this approach is intrinsically parallel and readily scalable to the monitoring of very large numbers of mRNAs" (p. 1679, col. 2, paragraph 2). One of ordinary skill in the art at the time the invention was made would have recognized the benefit provided by the hybridization technique taught by Lockhart and would have therefore been motivated to include hybridization of the target mRNA sample(s) to an oligonucleotide array with a reasonable expectation of success.

16. Claim 39 is rejected under 35 U.S.C. 103(a) as being unpatentable over Lockhart et al. (1996, Nature Biotech, vol. 14, p. 1675-1680) as applies to claims 37-38 and 42 above and further in view of Rampersad et al. (US Patent 5,830,712; November 1998) and further in view of Baker et al (US Patent 5,643,780; July 1997). Lockhart teaches hybridization to small, high-density oligonucleotide arrays, designed using a combination of photolithography and oligonucleotide synthesis (Abstract).

Lockhart teaches the limitations of claims 37, 38 and 42 as recited in the 102 rejection stated above. However, Lockhart does not teach the application of the technique to hybridization to blocking oligonucleotides.

Rampersad discloses a method for inactivating undesirable members in a sample which includes desirable and undesirable sequences in a mixture (Abstract).

With regard to claim 39, Rampersad discloses an embodiment of claim 38, wherein amplification of target mRNA is blocked by hybridization of one or more blocking molecules to

one or more target mRNA transcripts prior to extending said extendable primer with reverse transcriptase, wherein said one or more blocking molecules hybridize to said one or more target mRNA transcripts and block reverse transcription of said target mRNA transcripts (example 1, col. 5, lines 9-61, where RNA treated with the oligonucleotide blocker plus or minus oligonucleotide blocker and RNAse H were used as templates in reverse transcription reactions to generate cDNA, with the oligonucleotide blocker priming cDNA synthesis).

Regarding claim 39, Rampersad does not explicitly teach the targeting of globin mRNA molecules in the method of reducing highly or moderately expressed target sequences. Baker discloses that globin mRNA is one of the most highly expressed sequences in certain cell types (col. 5, lines 5-24).

It would have been prima facie obvious to one of ordinary skill in the art at the time the invention was made to have included the abundantly expressed globin gene taught by Baker in addition to the sequences that were targeted by Rampersad with a reasonable expectation for success. As taught by Rampersad, the term "undesired nucleic acids is used herein to denote nucleic acids in a sample that interfere with the use of the sample. Undesirable nucleic acids may include known or abundant members of a nucleic acid family that block the study, isolation or processing of desired novel or less abundant members of the same family" (col. 2, lines 30-34). As noted by Baker, "A common problem encountered is overabundance of a particular undesired message in the library. In many cells or tissues, a particular mRNA species represents the vast majority of the total mRNA. For example, abundant mRNAs, such as those encoding globin, immunoglobulins and ovalbumin may constitute as much as 50-90% of the total poly(A)+ cytoplasmic RNA isolated from certain cell types (col. 5, lines 5-12)." Considering the

abundance of expression of globin in certain cell types, it would have been prima facie obvious to include this target to decrease the abundance of this gene in the method of enrichment for those less highly expressed sequences in a complex mixture of nucleic acids. Furthermore, one of ordinary skill would have been motivated to apply the complex mixture of nucleic acids to the hybridization technique taught by Lockhart due to the benefit that "in addition to being sensitive, specific and quantitative, this approach is intrinsically parallel and readily scalable to the monitoring of very large numbers of mRNAs" (p. 1679, col. 2, paragraph 2). Given the teachings of both Baker and Rampersad and the hybridization assay taught by Lockhart, one of ordinary skill would have recognized the benefit of greater representation of less highly expressed genes in a complex mixture of nucleic acids and would therefore have been motivated to incorporate the globin sequence taught by Baker into the preparation of the nucleic acid molecules applied to the hybridization assay taught by Lockhart with a reasonable expectation for success.

17. Claim 40-41 are rejected under 35 U.S.C. 103(a) as being unpatentable over Lockhart et al. (1996, Nature Biotech, vol. 14, p. 1675-1680) as applies to claims 37-38 and 42 above and further in view of Mugasimangalam et al. (US Patent 6,544,741; September 2003), Baker et al (US Patent 5,643,780; July 1997) and Kempe et al. (US Patent 4,661,450; April 1987). Lockhart teaches hybridization to small, high-density oligonucleotide arrays, designed using a combination of photolithography and oligonucleotide synthesis (Abstract).

Lockhart teaches the limitations of claims 37-38 and 42 as recited in the 102 rejection stated above. However, Lockhart does not teach the use of blocking molecules.

Mugasimangalam discloses methods and materials for normalizing nucleic acids, using a technique called 'prime and kill' (col. 3, lines 44-51).

With regard to claim 40, Mugasimangalam discloses an embodiment of claim 38, wherein said one or more blocking molecules are peptide nucleic acids (col. 7, lines 48-51, where the killer primer can comprise peptide nucleic acids containing DNA analogs with amide backbone linkages).

With regard to claim 41, Mugasimangalam does not teach the application of the technique to globin target molecule. Kemp et al.teaches an embodiment of claim 39, wherein said one or more blocking molecules hybridize to a globin mRNA selected from the group consisting of alpha-1 globin, alpha-2 globin and beta globin (col. 27, lines 39-60, where beta globin is extracted from blood).

It would have been prima facie obvious to one of ordinary skill in the art at the time the invention was made to have included the abundantly expressed globin gene taught by Baker in addition to the sequences that were targeted by Mugasimangalam with a reasonable expectation for success. As taught by Mugasimangalam, "the methods and materials provided herein efficiently reduce the redundancy of highly expressed genes, and increase the relative amount of transcripts of rarely expressed genes" (col. 7, lines 5-13). As noted by Baker, "A common problem encountered is overabundance of a particular undesired message in the library. In many cells or tissues, a particular mRNA species represents the vast majority of the total mRNA. For example, abundant mRNAs, such as those encoding globin, immunoglobulins and ovalbumin may constitute as much as 50-90% of the total poly(A)+ cytoplasmic RNA isolated from certain cell types (col. 5, lines 5-12)." Considering the abundance of expression of globin in certain cell

types, it would have been prima facie obvious to include this target to decrease the abundance of this target gene in the method of enrichment for less highly expressed sequences in a complex mixture of nucleic acids. Furthermore, one of ordinary skill would have been motivated to apply the complex mixture of nucleic acids to the hybridization technique taught by Lockhart due to the benefit that "in addition to being sensitive, specific and quantitative, this approach is intrinsically parallel and readily scalable to the monitoring of very large numbers of mRNAs" (p. 1679, col. 2, paragraph 2). Given the teachings of both Baker and Mugasimangalam, and the hybridization assay taught by Lockhart, one of ordinary skill would have recognized the benefit of greater representation of less highly expressed genes in a complex mixture of nucleic acids and would therefore have been motivated to incorporate the abundant globin message taught by Baker into the preparation of the nucleic acid molecules applied to the hybridization assay taught by Lockhart with a reasonable expectation for success.

Claim 43 is rejected under 35 U.S.C. 103(a) as being unpatentable over Lockhart et al. (1996, Nature Biotech, vol. 14, p. 1675-1680) as applies to claims 37-38 and 42 above and further in view of Rampersad et al. (US Patent 5,830,712; November 1998), Baker et al (US Patent 5,643,780; July 1997) and further in view of Augello et al. (US Patent 6,602,718; August 2003). Lockhart teaches hybridization to small, high-density oligonucleotide arrays, designed using a combination of photolithography and oligonucleotide synthesis (Abstract).

With regard to claim 43, Augello discloses an embodiment of claim 37, wherein said nucleic acid sample is isolated from a blood sample that was collected in a container containing an RNA stabilizing agent selected from the group consisting of cationic compounds, detergents,

chaotropic salts, ribonuclease inhibitors, chelating agents, phenol, chloroform, acetone, alcohols, mercapto-alcohols, dithiothreitol (DTT) and mixtures thereof (col. 6, line 51 to col. 7 line 18). It would have been prima facie obvious to one of ordinary skill in the art at the time the invention was made to have included the abundantly expressed globin gene taught by Baker in addition to the sequences that were targeted by Rampersad with a reasonable expectation for success. As taught by Rampersad, the term "undesired nucleic acids is used herein to denote nucleic acids in a sample that interfere with the use of the sample. Undesirable nucleic acids may include known or abundant members of a nucleic acid family that block the study, isolation or processing of desired novel or less abundant members of the same family" (col. 2, lines 30-34). As noted by Baker, "A common problem encountered is overabundance of a particular undesired message in the library. In many cells or tissues, a particular mRNA species represents the vast majority of the total mRNA. For example, abundant mRNAs, such as those encoding globin, immunoglobulins and ovalbumin may constitute as much as 50-90% of the total poly(A)+ cytoplasmic RNA isolated from certain cell types (col. 5, lines 5-12)." Considering the abundance of expression of globin in certain cell types, it would have been prima facie obvious to include this target to decrease the abundance of this gene in the method of enrichment for those less highly expressed sequences in a complex mixture of nucleic acids. Furthermore, one of ordinary skill would have been motivated to apply the complex mixture of nucleic acids to the hybridization technique taught by Lockhart due to the benefit that "in addition to being sensitive, specific and quantitative, this approach is intrinsically parallel and readily scalable to the monitoring of very large numbers of mRNAs" (p. 1679, col. 2, paragraph 2). Given the teachings of both Baker and Rampersad and the hybridization assay taught by Lockhart, one of

ordinary skill would have recognized the benefit of greater representation of less highly expressed genes in a complex mixture of nucleic acids and would therefore have been motivated to incorporate the globin sequence taught by Baker into the preparation of the nucleic acid molecules applied to the hybridization assay taught by Lockhart with a reasonable expectation for success.

Furthermore, it would have been prima facie obvious to one of ordinary skill in the art at the time the invention was made to incorporate the teachings of Augello into the method taught by Rampersad with a reasonable expectation for success. As taught by Augello, upon evaluating the stabilization of nucleic acids obtained using the collection container with RNA stabilizers, "The real-time PCR results show that in the unpreserved EDTA blood, the transcript level decreases over time (indicated by the increasing Ct value in the TaqMan analysis) up to a degree of degradation after 7 to 10 days at which point the mRNA is no longer detectable. On the other hand the GAPDH mRNA in the preserved samples does not show any decrease in copy number" (col. 11, lines 26-33). Therefore, the inclusion of the RNA stabilizer and collection container taught by Augello provides the benefit of increased stability over time, allowing for analysis of RNA samples over a longer time frame. One of ordinary skill in the art would have recognized the benefit of blood collection in the presence of RNA stabilizers, who would have therefore been motivated to incorporate the teachings of Augello to the method taught by Rampersad with a reasonable expectation for success.

Conclusion

Any inquiry concerning this communication or earlier communications from the examiner should be directed to Stephanie K. Mummert, Ph.D. whose telephone number is 571-272-8503. The examiner can normally be reached on M-F, 8:30-5.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Gary Benzion can be reached on 571-272-0872. The fax phone number for the organization where this application or proceeding is assigned is 571-273-8300.

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Stephanie K. Mummert
Examiner
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